EFFECT OF NUTRIENT CONCENTRATION ON STEM ANATOMY OF EASTERN COTTONWOOD SEEDLINGS

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ABSTRACT

First-year seedlings of eastern cottonwood (*Populus deltoides* Bartr.) were grown under several different concentrations and combinations of N, P, K, Ca, and Mg. Fiber length, vessel length and width, and percentage of fibers and vessels in the seedling stems differed among treatments, but percentage of ray tissue did not. Fiber length showed a clear relationship to nutrient concentration; it increased to a maximum and then decreased as both N and K concentrations increased. Relationships between other morphological properties and the various nutrients were less clear-cut. Possibly because so little is known about the factors studied, the response surface design and analysis used did little to clarify the complicated relationships.

To a large extent the properties of wood depend on the structure produced as the wood is laid down. Control over how wood is produced theoretically lies in the hands of the forester, but practically only with the forester actively working with an intensively managed forest. With the gradual realization in the United States that the forester should be growing for the product rather than for the tree, the trend is toward more intensive care of the forest. This care includes fertilization of plantations and stands, aimed at faster production of more fiber with desirable qualities. Before widespread fertilization is undertaken, however, it is desirable to observe the effects of various nutrient levels on wood anatomy.

The majority of mineral nutrition-tree growth studies have concentrated on leaf color, root development, or stem size. However, several workers have examined the wood anatomy of relatively large trees following fertilization. While some, Gentle, Bamber, and Humphreys (1968), Jensen et al. (1964), and Posey (1964), found little or no effect on wood properties, others, such as Bhagwat (1967), Erickson and Lambert (1958), Takahashi (1968), and Zobel et al. (1961), found changes in wood anatomy associated with fertilizer application.

Working with seedlings produces data less directly applicable to forest conditions, but the ability better to control growing conditions enables the researcher to follow the effects of fertilizer application more precisely. Accordingly, work on seedlings has been reported by Davis (1949), Malavolta and Haag (1966), Lamb and Murphey (1968), and Foulger and Hacskaylo (1968), to name but a few.

That the mechanisms linking a particular nutrient to a visible function, e.g., cell length, are not yet understood should be no surprise because of the variations in anatomic changes reported to be associated with an element. The bulk of the evidence indicates that, particularly with high levels of fertilization, anatomic changes are associated with fertilizer application. There is no general agreement on the precise nature of the changes, however. This may be due in part to the variety of species examined and to the various concentrations and combinations of elements used. Generally, the reader of the literature in this area makes his choice and takes his chances. A major problem in examining nutrient effects on wood anatomy is the difficulty of disentangling the relationships when several levels of nutrients are varied simultaneously. In an attempt to solve this problem and still

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keep the size of the study within reasonable limits, a response surface design was employed.

MATERIALS AND METHODS

Variations in fiber length, vessel length and width, and in percentage of fiber, vessel, and ray tissue in eastern cottonwood (*Populus deltoides* Bartr.) seedlings grown under different N, P, K, Ca, and Mg concentrations and combinations are reported in this paper.

With seed from a single tree, cottonwood seedlings were grown in sand flats until they were about 5 cm tall, and then transplanted two to a pot into 5-gallon plastic pots filled with quartz sand. For the first 2 weeks after transplanting, the plants received only distilled water. Then one-third of the assigned nutrient supply was added, the remainder being added in two equal doses at 2-week intervals. The seedlings were harvested 30 days after addition of the full nutrient solution. During the study the pots were flushed four times daily and water was added when necessary to maintain a relatively constant concentration of nutrients in each pot.

TABLE 1. *Combinations of nutrient levels used for eastern cottonwood seedlings*

	Nutrient levels, ppm				
No.	N	P	K	Ca	Mg
1	27	6	24	18	109
2	199	6	24	18	12
3	27	40	24	18	12
4	199	40	24	18	109
5	27	6	218	18	12
6	199	6	218	18	109
7	27	40	218	18	109
8	199	40	218	18	12
9	27	6	24	162	12
10	199	6	24	162	109
11	27	40	24	162	109
12	199	40	24	162	12
13	27	6	218	162	109
14	199	6	218	162	12
15	27	40	218	162	12
16	199	40	218	162	109
17	10	15	73	54	36
18	538	15	73	54	36
19	74	2	73	54	36
20	74	108	73	54	36
21	74	15	8	54	36
22	74	15	654	54	36
23	74	15	73	6	36
24	74	15	73	486	36
25	74	15	73	54	4
26	74	15	73	54	327
27	74	15	73	54	36

CELL MEASUREMENTS

A sample, 5 cm long, was cut from the middle of the larger stem in each pot. All samples were initially stored frozen, then thawed, and stored in FAA solution (50 cc 95% ethyl alcohol, 5 cc glacial acetic acid, 10 cc 40% formaldehyde, 35 cc water). A portion, about 15 mm long, was taken from the upper half of each sample and the wood macerated in an acetic acid-hydrogen peroxide equal-volume mixture. Temporary slides of the macerated material were used to make measurements at 110× magnification of fiber length, vessel length, and vessel width. Analysis of measurements from these slides showed that 30 fiber length measurements and 10 vessel length and 10 width measurements per sample were needed to get a ±5% estimate (95% confidence level) of the mean value

A cross-section face was cut on the lower portion of the sample with a freezing microtome. Measurements of fiber, vessel, and ray frequency were made at 176× magnification on each cross section with an integrating eyepiece; 10 randomly selected fields were examined per specimen.

EXPERIMENTAL DESIGN AND ANALYSIS

Five levels of N, P, K, Ca, and Mg were used in the study. Rather than a complete factorial design requiring 3125 nutrient level combinations, 27 combinations were selected that could be analyzed by the response surface methods described by Box and Hunter (1957). Details of this particular design and its analysis can be found in publications by Hader et al. (1957) and by Cochran and Cox (1957), while Phares (1967) recently discussed their application to nutrient requirement studies. The actual nutrient levels used in each of the 27 treatments are shown in Table 1. The nutrient levels were coded linearly as –2, –1, 0, +1,

TABLE 2. *Coding of nutrient levels*

Code level	Basic levels, ppm				
	N	P	K	Ca	Mg
−2	10	2	8	6	4
−1	27	6	24	18	12
0	74	15	73	54	36
1	199	40	218	162	109
2	538	108	654	486	327

and +2, using the logarithms of the concentrations; the coded values and concentrations are shown in Table 2.

The 27 treatment combinations were planned to enable the results of the study to be examined by a second-order response surface of the form

$$Y = \beta_o + \sum_{i=1}^{5} \beta_i X_i + \sum_{i=1}^{5} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{5} \sum_{j=1}^{5} \beta_{ij} X_i X_j,$$

where Y = the response variable

X_i = the coded level of nutrient i

and β = the regression coefficients, estimated from the experimental data.

The aim of fitting this type of surface was to determine whether the response variable was related to the level of one or more of the nutrients and to determine approximately the form of this relationship. The technique is strictly exploratory but is often useful when dealing with a large number of factors about which little is known.

The original choice of nutrient levels was made to simplify both the estimation of regression coefficients and the testing of selected sets of coefficients. Unfortunately, all replications of treatments 2, 14, and 19 were lost and with them went much of the simplicity of fitting and testing.

There were five distinct steps in the analysis for each of the following dependent variables: fiber length; percentage of fibers, vessels, and rays; and vessel length and width. First, an analysis of variance tested the variation among the 24 treatments against the variation between the three observations within each treatment. In the second step, the full 21-term response surface described was fitted. The sum of squares associated with this regression, the deviations from regression, and all of the fitted regression coefficients were tested, again using analysis of variance. Third, a series of five 15-term equations was fitted and tested, the equations being formed by deleting all terms involving N (or P, or K, etc.) from the original 21-term model. Examining results of the previous steps gave some indication of which terms in the original model had the greatest predictive value for a given dependent variable. Equations involving these terms were fitted and tested in the fourth step. Finally the resulting equations were examined for implications of the effects of various nutrients and optimum levels.

It should be noted that the probability levels associated with this "hunt and peck" system may not be equal to the levels selected for the test. Numerous tests are being made, mostly of hypotheses suggested by the same data on which the test is made. The results are informative in exploratory work such as this, but the cooking process calls for a leavening of suspicion.

RESULTS

Fiber length

Analysis of the observations suggested that fiber length was most closely associated with the levels of N and K. The fitted equation relating fiber length to all N and K terms was:

$$\begin{aligned}\text{Fiber length} = {} & 0.531 - 0.026\,N \\ & + 0.007\,K + 0.011\,NK \\ & - 0.010\,N^2 - 0.016\,K^2. \qquad (1)\end{aligned}$$

This equation, which accounts for 79% of the variation among treatments, indicates that fiber length was maximized in the vicinity of N = −1.455 and K = −0.281 coded values, or N = 17.2 ppm and K = 53.1 ppm. The predicted fiber length at this point was 0.55 mm, as compared with 0.44 mm at the highest concentrations of N and K, 538 ppm and 654 ppm respectively. This may be compared with an average fiber length for eastern cottonwood of 1.12 mm reported by Bergman (1949). The gen-

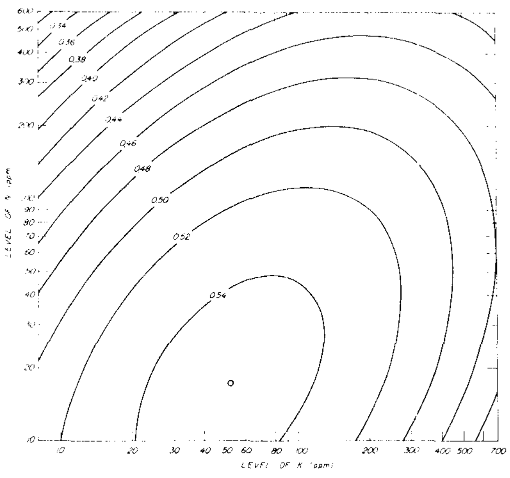

FIG. 1. Variation in fiber length in mm with change in ppm of N and K.

eral surface developed by the equation is shown in Fig. 1, while average fiber lengths by treatment are included in Table 3.

In interpreting these results, it should be realized that a fitted equation is, at best, but a mathematical approximation of the observed facts within the test region. The exact location of a maximum point may vary somewhat, depending on the form of the equation fitted. Further, when a maximum occurs near the test region boundary, as it does for N, even the existence of a true maximum may be doubtful.

Equation 1 developed for fiber length expresses a relationship between the coded levels of N and K and the fiber length averaged over all experimental levels of

TABLE 3. *Response of eastern cottonwood seedlings to nutrient treatments*

Treatment number	Maceration			Per cent cross section occupied by—			Seedling characteristics	
	Fiber length (mm)	Vessel length (mm)	Vessel width (mm)	Fiber	Vessel	Ray	Total dry weight, (g)	Total height, (cm)
1	0.510	0.425	0.059	59.9	29.6	10.5	11.02	93
2[a]	—	—	—	—	—	—	1.69	18
3	0.567	0.390	0.047	64.1	27.2	8.7	16.88	127
4	0.460	0.361	0.049	53.2	37.4	9.4	12.22	68
5	0.510	0.410	0.056	61.9	28.4	9.7	12.58	94
6	0.502	0.380	0.046	58.4	32.3	9.3	1.69	30
7	0.544	0.439	0.057	61.5	26.1	12.4	17.16	121
8	0.468	0.379	0.067	60.5	29.3	10.2	27.46	119
9	0.546	0.407	0.063	57.0	31.7	11.3	14.80	107
10	0.466	0.391	0.050	59.0	30.9	10.1	3.67	45
11	0.525	0.412	0.056	57.1	31.3	11.6	11.31	104
12	0.449	0.371	0.052	45.9	40.8	13.3	11.74	73
13	0.535	0.415	0.054	58.8	31.8	9.4	11.56	105
14[a]	—	—	—	—	—	—	0.53	13
15	0.530	0.441	0.063	59.5	29.0	11.5	17.24	123
16	0.521	0.391	0.065	60.0	30.2	9.8	54.92	158
17	0.534	0.419	0.046	61.1	28.3	10.6	7.63	86
18	0.444	0.325	0.055	57.2	28.3	14.5	22.05	84
19[a]	—	—	—	—	—	—	0.23	12
20	0.540	0.435	0.062	58.3	31.7	10.0	3.43	153
21	0.455	0.369	0.055	54.0	33.3	12.7	16.72	105
22	0.479	0.369	0.052	53.6	32.7	13.7	32.18	133
23	0.511	0.408	0.051	59.2	29.4	11.4	24.27	118
24	0.533	0.398	0.057	58.0	33.0	9.0	35.29	153
25	0.560	0.408	0.057	54.8	33.6	11.6	41.69	151
26	0.503	0.378	0.055	55.1	33.6	11.3	32.60	133
27	0.532	0.401	0.059	57.8	31.8	10.4	31.72	140
Average	0.509	0.397	0.056	57.8	31.3	10.9		

a = No data, replicates missing.

P, Ca, and Mg. It is not necessarily true that the fiber length for a given N and K combination would be the same if P, Ca, or Mg, or all three were wholly absent, or if they were present at levels other than those used here.

Percentage of fibers

Only the observations on fiber length displayed a clearly definable relationship to the nutrient levels. In the following paragraphs it will become apparent that, although a number of significant regressions appeared, the complexity of the interactions made it impossible to produce simple, clear-cut relationships.

Percentage of fibers was obtained from observations on the cross-section surface of each stem. The following equation showed the strongest relationship between percentage of fibers and nutrient levels:

$$\begin{aligned}\text{Percentage of fibers} = {} & 56.886 - 1.686\,\mathrm{N} - 1.471\,\mathrm{Ca} \\ & + 1.329\,\mathrm{N}\cdot\mathrm{Mg} + 1.965\,\mathrm{P}\cdot\mathrm{K} \\ & + 2.436\,\mathrm{Ca}\cdot\mathrm{Mg} + 0.745\,\mathrm{N}^2. \quad (2)\end{aligned}$$

Although a significant portion (70%) of the variation among treatments in percentage of fibers was associated with this equation, the number and variety of interaction terms involved make it difficult to draw any firm conclusions on the effects of the five nutrients. Each nutrient appears in at least one term of the equation. It seems reasonable that N influenced the percentage of fibers, since three terms of the equation involve N. It is impossible to make any simple statement about how percentage of fibers is affected by the level of N since the N effect varies with the level of Mg.

There is, also, some evidence of a Ca effect. Throughout the analysis, it was difficult to separate the effects of Ca and K. Often one or more Ca terms could be substituted for K terms with a change in sign and little loss in precision. Thus what appears to be a Ca effect may be, in fact, a K effect or vice versa. Ca is necessary for the hydrolysis of adenosine triphosphate (ATP) by some enzymes, and since K may also be required for the proper function of certain ATP-ases, the two elements may be able to substitute for each other to a degree.

Nothing definite can be said about the effects of P and Mg. Since P appears only in the $\mathrm{P}\cdot\mathrm{K}$ interaction term, this may merely reflect a chance modification of the relationship of fiber per cent to the level of K. Whenever Mg appeared in the analyses it usually made a substantial contribution to the precision of the equation, but it appeared invariably as an interaction term, never alone. It is known that Mg is required by a large number of enzymes involved in phosphate transfer, and possibly this is being reflected by its appearance as an interaction term.

Percentage of vessels

The best equation ($R^2 = 0.82$) found for predicting percentage of vessels was

$$\begin{aligned}\text{Percentage of vessels} = {} & 32.286 + 1.212\,\mathrm{N} + 1.404\,\mathrm{Ca} \\ & + 1.299\,\mathrm{N}\cdot\mathrm{P} - 1.993\,\mathrm{P}\cdot\mathrm{K} \\ & - 1.697\,\mathrm{Ca}\cdot\mathrm{Mg} - 1.043\,\mathrm{N}^2. \quad (3)\end{aligned}$$

As would be expected with a high correlation between percentages of fibers and vessels, results for these two variables were very similar. Further, where a given term appeared in both equations, the coefficients invariably were opposite in algebraic sign. As with percentage of fibers, nitrogen seemed to have the greatest effect, with calcium having a lesser effect than nitrogen.

Vessel length

A large proportion, 70%, of the variation between treatments was accounted for by the equation

$$\text{Vessel length} = 0.386 - 0.021\,\mathrm{N} + 0.012\,\mathrm{P}^2. \quad (4)$$

This equation indicated that vessel length decreased as the N level increased, a trend similar to that observed for fiber length at the higher N levels. The equation is less satisfactory in its indication that minimum vessel length occurs when $\mathrm{P} = 0$ (i.e., $\mathrm{P} = 15$ ppm) and increases as P either decreases or increases. Average vessel length for all treatments was 0.40 mm, compared with 0.64 mm average length reported in mature eastern cottonwood (Bergman 1949).

As noted, either the form of the equation or the method of coding the variables sometimes creates artificial peaks or valleys. Rather than accepting that a minimum occurs at $P = 15$ ppm, it is more realistic to assume that vessel length changes very little below $P = 15$ ppm. Above that concentration, vessel length would increase with increasing P levels.

Vessel width

The most useful variables for vessel width prediction were P, N·P, and P·K in the equation

$$\text{Vessel width} = 0.055 + 0.002\,P + 0.003\,N \cdot P + 0.005\,P \cdot K. \qquad (5)$$

Sixty per cent of the variation among treatments was associated with this equation. The equation suggests that P plays an important role in vessel width development. Two of the three terms involving N that were in the equation for percentage of vessels are not in the vessel width equation. This could mean that N was affecting the number of vessels or that N was indirectly influencing the percentage of area in vessels by its effect on the width or number of fibers.

Percentage of ray tissue

Neither the differences among treatments nor the variation associated with the full response surface was significant for the percentage of cross section occupied by rays. Although the value of any fitted equations is questionable under these circumstances, it seemed desirable to examine the terms in some of the better fitting regression equations. One of these is

$$\text{Percentage of rays} = 11.031 + 0.041\,N - 0.722\,N \cdot Mg + 0.487\,K^2. \qquad (6)$$

The N and N·Mg terms in equation (6) appear with signs reversed in equation (2) for percentage of fibers. This suggests that their effect on the amount of ray tissue is probably a reflection of their more marked effect on the area occupied by fibers. The effect of the K^2 term on percentage of rays is not clear, however. Only 22% of the variation among treatments was associated with this equation.

Relationship of anatomy to plant growth

An analysis was made to investigate whether or not a relationship existed between the anatomic variables and plant height or total dry weight of the plant. Vessel width showed a positive correlation with both plant height ($r = 0.57$) and total dry weight ($r = 0.53$). Fiber length was positively correlated only with total plant height. Vessel length and the cross-sectional areas made up of fibers, vessels, or ray tissue did not show an association with plant height or total dry weight.

CONCLUSIONS

In this investigation, the only relatively uncomplicated association established was between fiber length and N and K. Equations for vessel width and for percentage of vessels and percentage of fibers ascribed considerable importance to the P·K term. The equation for percentage of vessels and the equation for percentage of fibers both contained the terms N, N^2, Ca, P·K, and Ca·Mg. These combinations suggest that N, K, and P, especially P, are of prime importance in their effect on vessel width, whereas N and P are important in the determination of vessel length. Although response surface designs and methods of analysis are powerful tools, these analyses showed that they do not necessarily provide clear-cut pictures when employed on factors about whose behavior little is known.

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